Subcellular Localization and Nuclear Import of Maize Fine Streak Virus and *Maize Mosaic Virus* Proteins

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Introduction

Maize fine streak virus (MFSV) and *Maize mosaic virus* (MMV) are members of the genus *Nucleorhabdovirus* in the family *Rhabdoviridae*. Plant rhabdoviruses are divided into two genera, *Nucleorhabdovirus* and *Cytorhabdovirus*. Nucleorhabdoviruses assemble at inner nuclear envelopes, whereas cytorhabdoviruses assemble at cytoplasmic membranes.

The MFSV genome encodes seven proteins in the gene order 3′-N-P-3-4-M-G-L-5′, and the MMV genome encodes six proteins in the order 3′-N-P-3-M-G-L-5′ **(Fig. 1)**. Nucleorhabdoviruses assemble in the nuclei of their plant and insect hosts, and therefore nuclear import of viral proteins is critical to complete morphogenesis. Nuclear import of nuclear localization signal (NLS)-containing proteins is mediated by Importin α and β. Importin α binds NLS-containing protein, and this heterodimer subsequently binds Importin β. The tripartite complex then docks to the nuclear pore followed by translocation into the nucleus. **We show that the MFSV N and P complex colocalizes to the nucleolus and is dependent on Importin α for nuclear import.** This is the first demonstration that Importin α is involved in nuclear import of rhabdoviral proteins in plant cells.

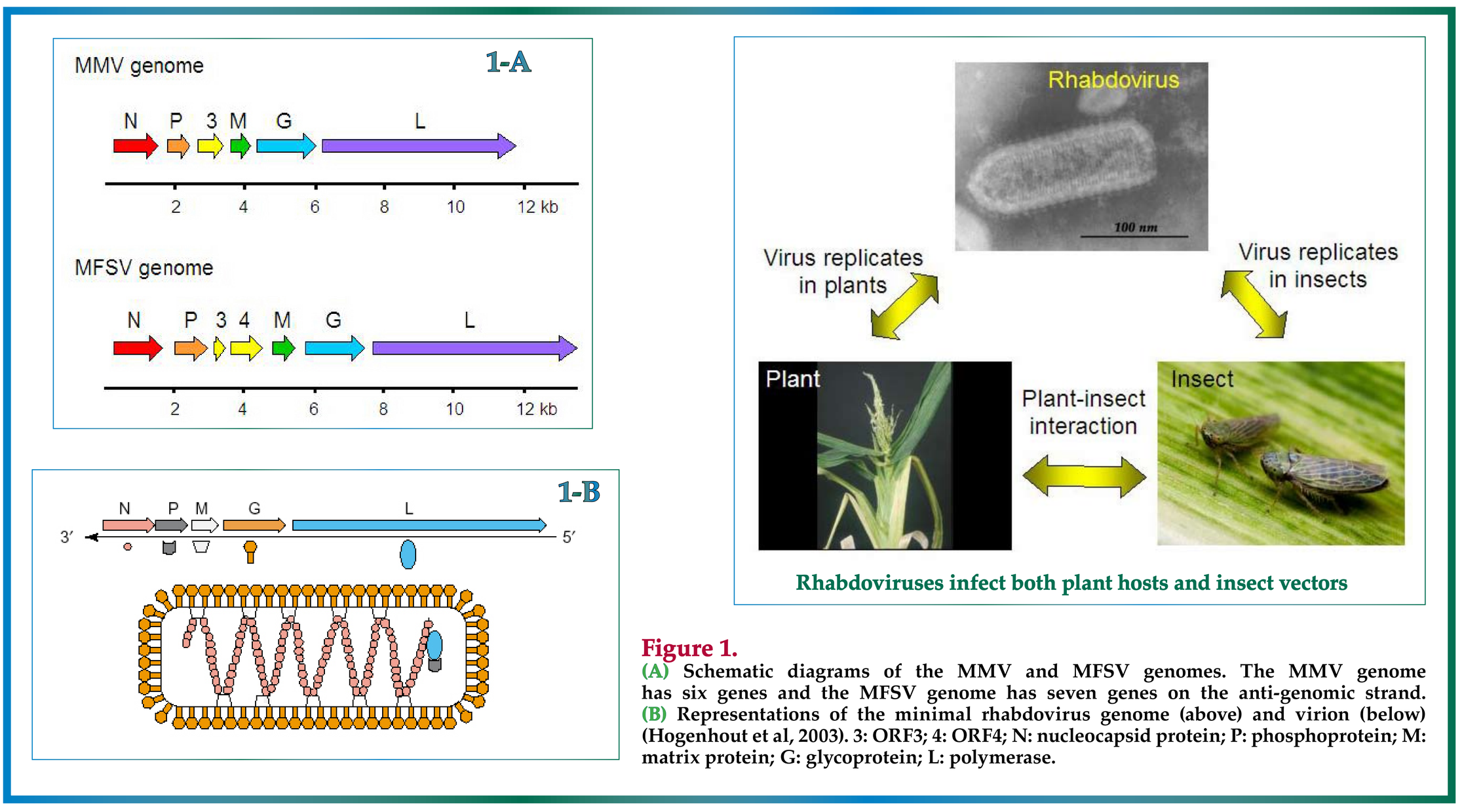

Figure 1.
(A) Schematic diagrams of the MMV and MFSV genomes. The MMV genome has six genes and the MFSV genome has seven genes on the anti-genomic strand. **(B)** Representations of the minimal rhabdovirus genome (above) and virion (below) (Hogenhout et al, 2003). 3: ORF3; 4: ORF4; N: nucleocapsid protein; P: phosphoprotein; M: matrix protein; G: glycoprotein; L: polymerase.

The goals of this study are to

1. Determine the cellular localization of MFSV proteins in *Nicotiana benthamiana* leaves.
2. Compare the cellular localization of MFSV and MMV proteins in *N. benthamiana* leaves.
3. Examine whether nuclear import of MFSV and MMV proteins is dependent on Importin α.

Materials and methods

1. Fluorescent-protein fusions of the MFSV N, P, 3, 4, and M proteins were agroinfiltrated into *N. benthamiana* leaves.
2. Fluorescent-protein fusions of the MMV N, P, 3, and M proteins were agroinfiltrated into *N. benthamiana* leaves.
3. Fluorescent-protein fusions of the MFSV N and P proteins were coinfiltrated into *N. benthamiana* leaves in which the expression of Importin α was silenced by virus-induced gene silencing (VIGS).

Results

1. The cellular localization of MFSV proteins. The MFSV N protein localized to the nucleus, whereas the MFSV P protein was distributed throughout the cell **(Fig. 2)**. When the MFSV N and P proteins were coinfiltrated, both proteins relocalized to the nucleolus of cell **(Fig. 3)**. This is in contrast to the N and P proteins of another nucleorhabdovirus *Sonchus yellow net virus* (SYNV) that localized to another part of nucleus (data not shown). The N and P protein relocalization was specific to cognate proteins of each virus. The MFSV 4 and M proteins localized to the nuclei, and the MFSV 3 protein accumulated in punctate loci in the cytoplasm **(Fig. 4)**. The localization of the MFSV N, 4, and M proteins in plant cell nuclei is consistent with the presence of NLSs.

2. The cellular localization of MMV proteins. The MMV N, 3, and M proteins were distributed throughout the cells, whereas the MMV P protein localized to the nucleus **(Fig. 5A)**, consistent with the presence of NLS in the MMV P protein, and the absence of NLS in the MMV N, 3, and M proteins. Upon coinfiltration of the MMV N and P proteins, both proteins were redirected to the cytoplasm **(Fig. 5B)**. The cellular localization of MMV proteins is different from that of MFSV, even though both MMV and MFSV are nucleorhabdoviruses and have a common plant host, maize.

3. The nuclear import of the MFSV N and P complex is dependent on Importin α. The MFSV N protein was distributed throughout the cells in Importin α1 and Importin α2 silenced, but not control plants. The MFSV P protein was distributed throughout the cells in silenced and control plants **(Fig. 6A)**. The MFSV N and P complex in silenced plants relocalized to other parts of the cell as opposed to the nucleolus in control plants **(Fig. 6B)**. The results show that the import of the MFSV N and P complex into the nucleolus is dependent on Importin α.

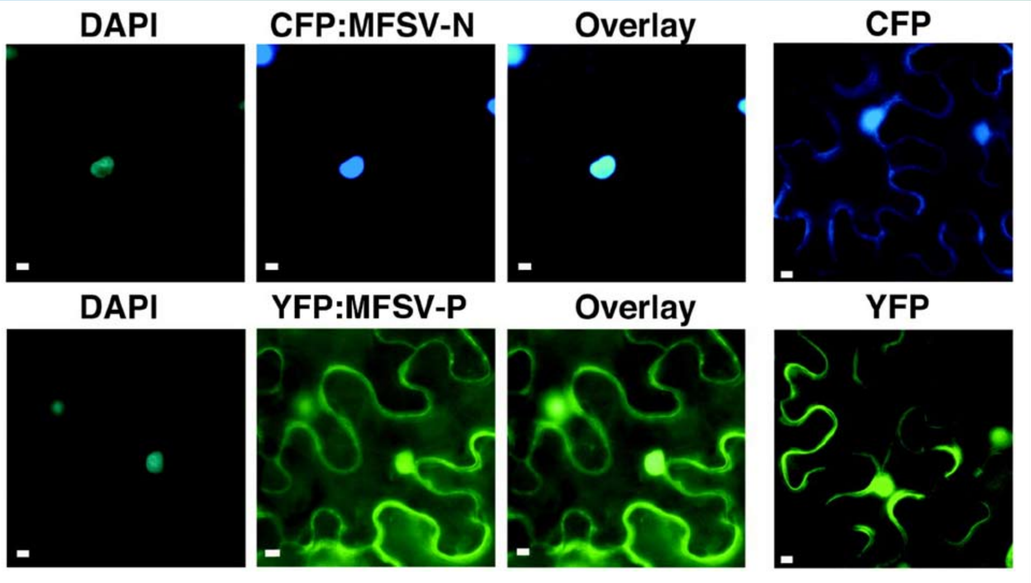

Figure 2.
The MFSV N protein localizes to the nucleus, whereas the MFSV P protein is distributed throughout the cell. Cellular views of localizations of CFP:MFSV-N to the nucleus, and YFP:MFSV-P throughout the cell are shown. Infiltrations of unfused CFP and YFP were included as negative controls. DAPI (4′-6-diamidino-2-phenylindole, dihydrochloride) stained nucleic acids and was used to determine the positions of nuclei in *Nicotiana benthamiana* cells. Bars = 5 μm.

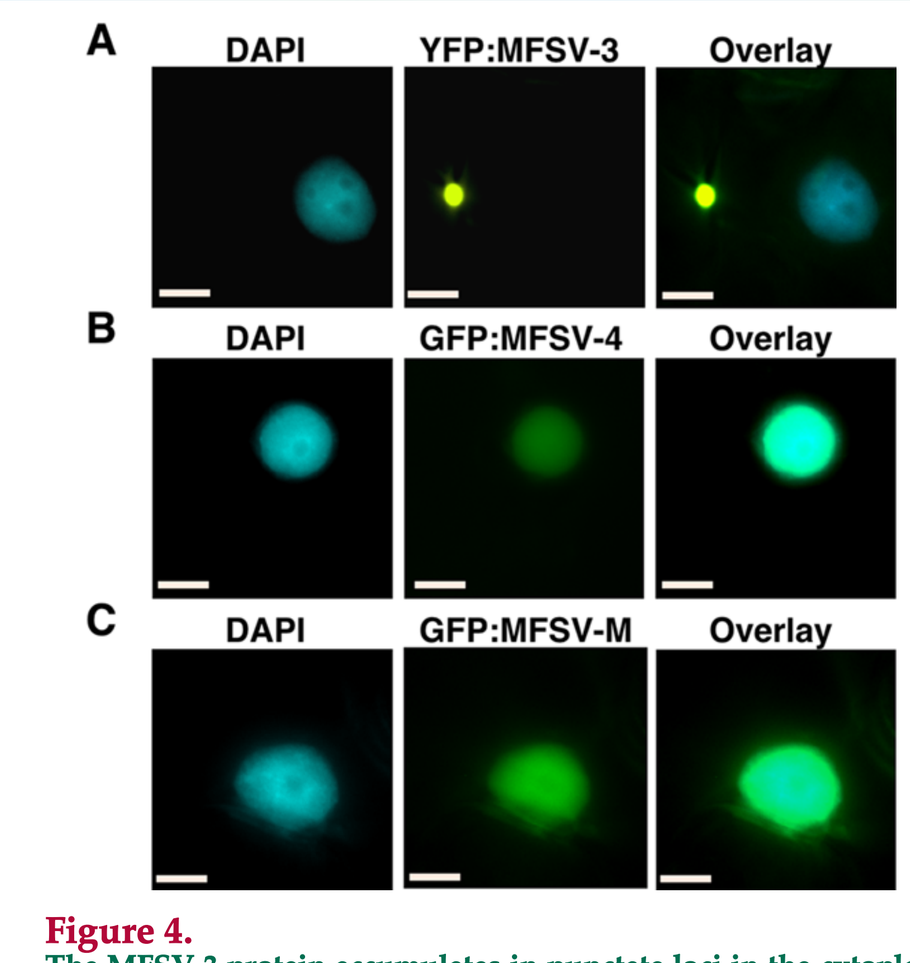

Figure 4.
The MFSV 3 protein accumulates in punctate loci in the cytoplasm, whereas the MFSV 4 and M proteins target the nuclei. (A) Accumulation of YFP:MFSV-3 in punctate locus in the cytoplasm. (B) Nuclear localization of GFP:MFSV-4. (C) Nuclear localization of GFP:MFSV-M. Bars = 5 μm.

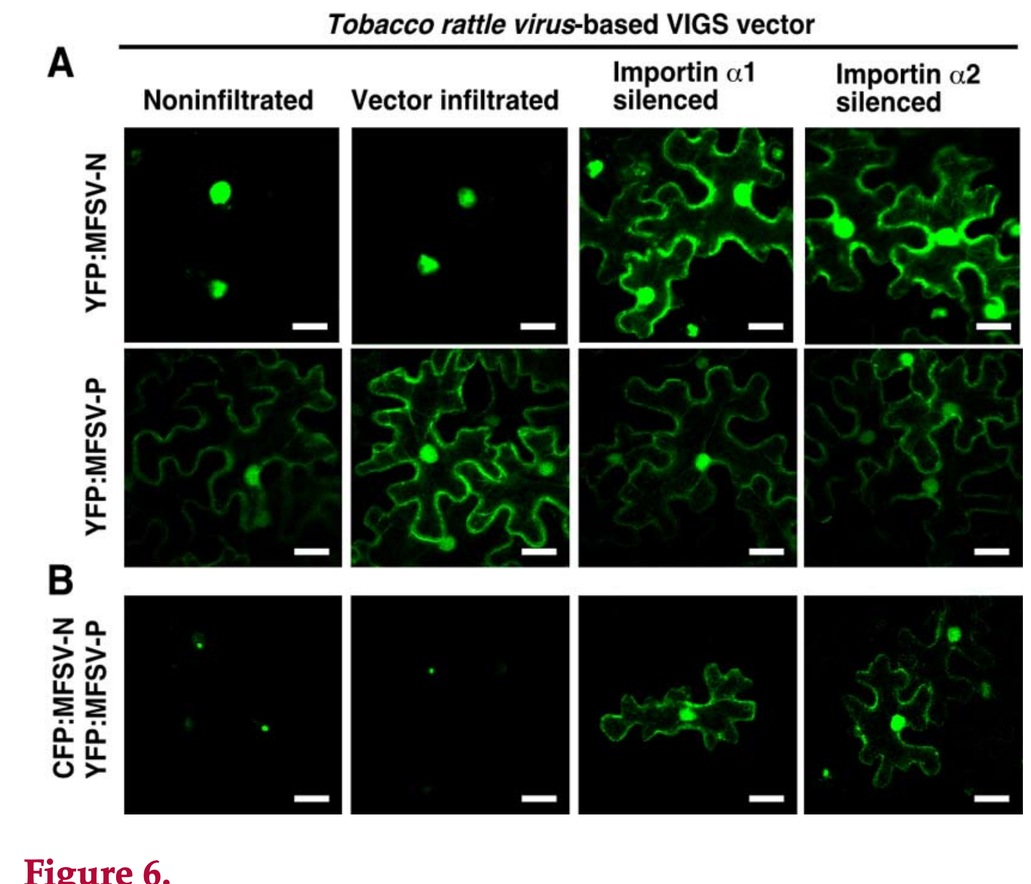

Figure 6.
The nuclear import of the MFSV N protein and the MFSV N and P complex is dependent on Importin α. (A) YFP:MFSV-N was distributed throughout the cells in Importin α1 and Importin α2 silenced, but not control leaves. YFP:MFSV-P was distributed throughout the cells in both silenced and control leaves. (B) CFP:MFSV-N and YFP:MFSV-P were distributed throughout the cells in Importin α1 and Importin α2 silenced, but not control leaves. See also Fig. 3B for localization of the MFSV N and P complex to plant cell nucleolus. Bars = 20 μm.

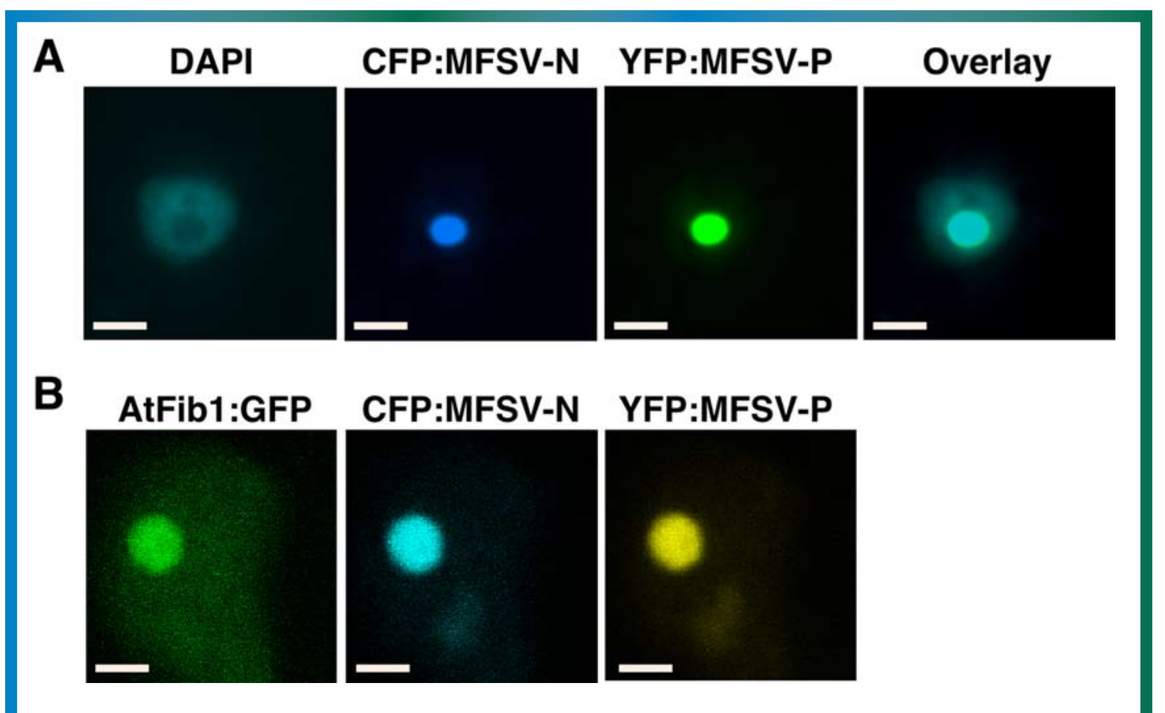

Figure 3.
Interaction of the MFSV N and P proteins results in localization of both proteins to the nucleolus of plant cell. (A) Epifluorescence micrographs of coinfiltrated CFP:MFSV-N and YFP:MFSV-P targeting the nucleolus. (B) Confocal micrographs of the colocalization of AtFib1:GFP, CFP:MFSV-N, and YFP:MFSV-P in the nucleolus. *Arabidopsis thaliana* Fib1 (AtFib1) is known to target the nucleolus of plant cell (Barneche et al, 2000). Bars = 5 μm.

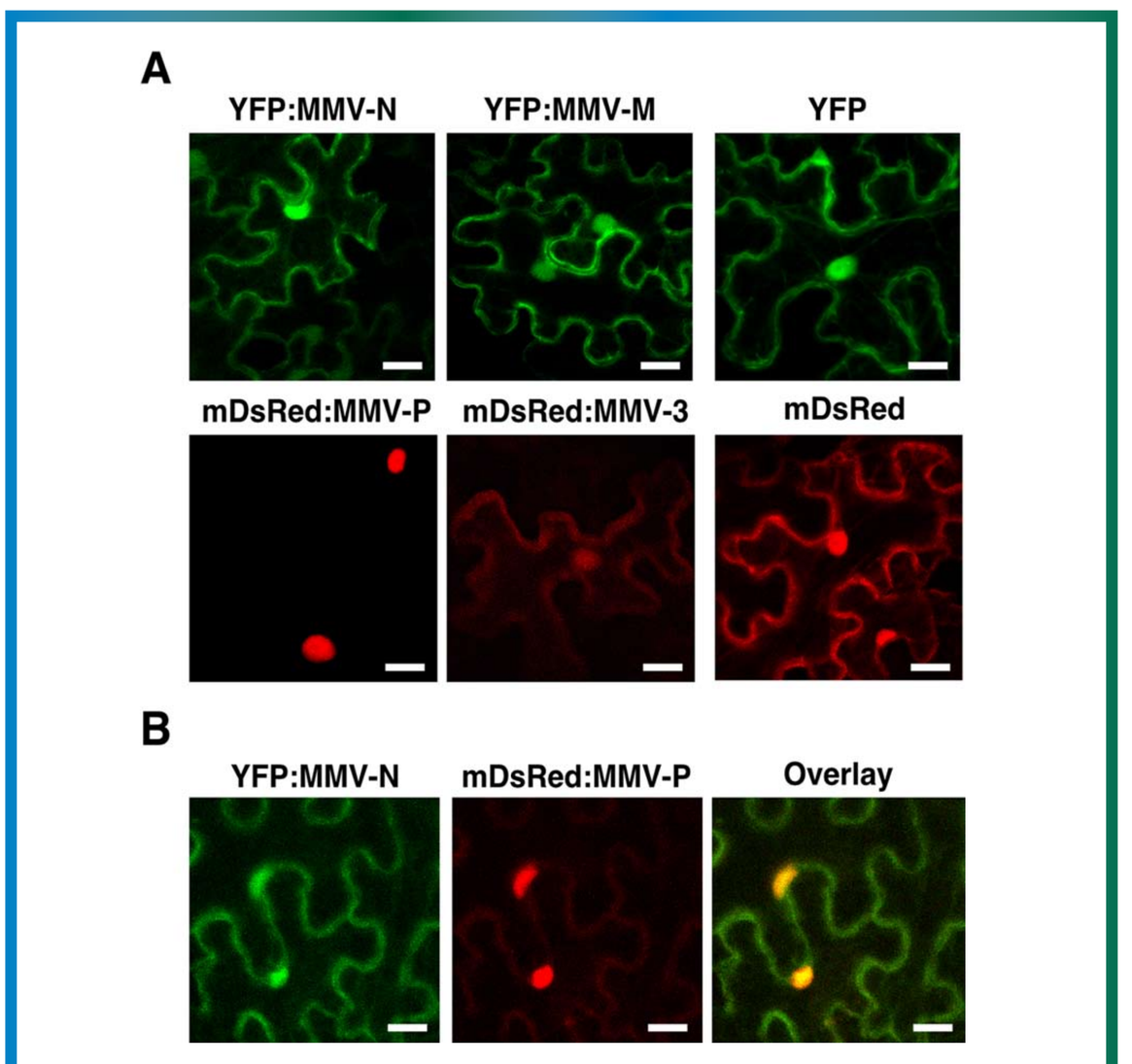

Figure 5.
The MMV N, 3, and M proteins are distributed throughout the cells and the MMV P protein localizes to the nucleus, whereas the coinfiltrated MMV N and P proteins relocalize throughout the cell. (A) YFP:MMV-N, monomeric DsRed (mDsRed):MMV-3 and YFP:MMV-M were distributed throughout the cells, whereas mDsRed:MMV-P localized to the nucleus. (B) Coinfiltrated YFP:MMV-N and mDsRed:MMV-P were redirected to the cytoplasm. Bars = 20 μm.

Major Conclusions

1. The MFSV N and P proteins target nucleolus, whereas the SYNV N and P proteins don't.
2. The localization of the MFSV and MMV proteins in plant cells is different even though both viruses are nucleorhabdoviruses and infect maize.
3. The targeting of the MFSV N and P complex to plant cell nucleolus is dependent on importin α.

Future Directions

1. The MFSV 4 and M proteins and the MMV P protein also have NLSs and target the nuclei (Figs. 4 and 5). We will investigate whether nuclear import of these proteins is dependent on Importin α.
2. Rhabdoviruses have diverse host ranges, e.g. MFSV replicates in its plant hosts and insect vectors. We hypothesize that rhabdoviruses interact with conserved proteins/pathways in plants and insects and this enables them to infect diverse hosts. Our next step is to test whether the MFSV N and P proteins also interact with Importin α of insect cells.

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Acknowledgements

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